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Differential susceptibility to tobacco smoke: possible mechanisms

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Prevalence data indicate that in 1987, 32% of men smoked and 27% of women smoked (United States Department of Health and Human Services, 1989). In addition, nearly 40% of American homes contain at least one cigarette smoker. Tobacco smoke is therefore one of the most prevalent personal and environmental exposures today and the health effects of tobacco smoke exposure will continue to be important for at least the next several decades. Understanding factors responsible for differential susceptibility to respiratory diseases caused by tobacco smoke will be critically important in reducing the burden these diseases place on individuals and on society.

The main themes of this report are that biological variability of the non-neoplastic acute and chronic responses to tobacco smoke is well established, but that traditional tools of toxicology and epidemiology have provided little understanding of the basis for this variability. Inhalation challenge studies using inbred strains of mice represent a powerful new approach to use in understanding differential susceptibility to the non-neoplastic effects of tobacco smoke. This report will summarize evidence for biological variability in the acute and chronic responses to tobacco smoke exposure and provide examples of recent research, which demonstrate allelic variation in the host response to relevant toxins or agonists.

Tobacco smoke is a complex aerosol comprised of nicotine, the particle phase, and vapour phase. Mainstream tobacco smoke is the smoke which smokers inhale, while environmental tobacco smoke is the smoke that nonsmokers inhale. Environmental tobacco smoke (ETS) is the combination of sidestream tobacco smoke (which comes from the burning end of the cigarette) and exhaled mainstream smoke. Most measured pollutants in both the particulate and vapour phases of ETS derive predominantly from sidestream tobacco smoke. In one study, the sidestream:mainstream ratio per cigarette was 3.3 for the number of particulates, 1.8–2.8 for nicotine, 2.6 for total phenols, 46 for ammonia and 3.6 for nitrogen oxides (Hoegg, 1972). The components of mainstream smoke were reduced 50–99% when mainstream smoke was inhaled into the lungs. When tobacco smoke is bubbled through medium, large quantities of oxidants including hydrogen peroxide are detected (Nakayama *et al.*, 1984) and the vapour phase also contains irritating aldehydes such as acrolein and formaldehyde. For smokers, the dominant exposure is through mainstream smoke, while for non-smokers sidestream smoke dominates the exposure profile. Smokers clearly are exposed to greater quantities of smoke than nonsmokers, but the dose–response relationships for smoke may be different for the two groups. For example, symptoms of tobacco smoke rhinitis were significantly more common among non-smokers (Annesi *et al.*, 1987). This speculation is also based on the recognition that, in the occupational setting, susceptible individuals often self-select away from an exposure.

The acute response to tobacco smoke is a neuro-inflammatory response mediated through stimulation of chemosensitive neurones. Understanding the basis for biological variability in the acute response to tobacco smoke may also allow an understanding of biological variability to other irritants. Animal studies show that the mucosal response to tobacco smoke is mediated via activation of c-fibre neurones by the organic vapour phase of tobacco smoke. This is an interesting contrast to previous studies which have pointed to the pathogenetic importance of the particulate phase of smoke in inflammatory responses with chronic exposure. In a series of studies, animals were exposed to tobacco smoke and nose wipings used as the measure of the acute inflammatory response for guinea pigs (Lundblad & Lundberg, 1984). Evans blue dye extravasation was used as the measure of response for similar studies in rats (Lundberg *et al.*, 1983).

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Nasal inflammation was absent with clean air exposure and significantly elevated following tobacco smoke exposure. The response persisted with exposure to the organic vapour phase alone or with exposure to nicotine-free cigarettes. Inflammation also occurred with vagal stimulation in the presence of atropine and with administration of substance P. The response to smoke was abolished by pretreatment with local anesthesia, systemic capsaicin, or substance P antagonists (Lundberg *et al.*, 1984). These data indicate that the acute response to tobacco smoke in these animals occurred through activation of capsaicin sensitive nerves which release substance P.

The acute symptomatic response to environmental tobacco smoke in humans shows evidence for biologic variability. Seventy-seven healthy nonsmoking young adults completed a questionnaire asking about a history of mucosal and respiratory symptoms following environmental tobacco smoke exposure. Eighty-two percent reported a history of eye irritation, 18% reported nasal irritation and 36% reported one or more rhinitis symptoms, including nasal congestion, sneezing, postnasal drip or rhinorrhea (Bascom, 1991a). Annesi *et al.* (1987) reported a prevalence of 5% of sneezy or runny nose with tobacco smoke exposure among a group of 332 men (both smokers and nonsmokers).

The acute physiological response to tobacco smoke also shows evidence for biological variability. A heterogeneous pulmonary function response to mainstream tobacco smoke in healthy adults (Nadal & Comroe, 1961) and to ETS in asthmatics (Stankus *et al.*, 1988) has been demonstrated. A heterogenous nasal congestive response has also been demonstrated (Bascom, 1991a). The historically ETS-sensitive subjects showed a significant increase in nasal resistance following controlled challenge to a brief, high level of sidestream tobacco smoke (45 ppm CO, 15 min) while the historically ETS-nonsensitive did not. Exposure to the organic vapour phase alone also caused nasal congestion in ETS-sensitive subjects, but not ETS-nonsensitive subjects (Willes *et al.*, 1991). This is evidence for biologic variability in the acute response to the organic vapour phase component of tobacco smoke.

Additional studies have indicated that increased upper airway responsiveness to tobacco smoke is not due to sensitive subjects receiving an increased dose of smoke since there was no correlation between the magnitude of the nasal resistance change and the change in urinary cotinine:creatinine ratio following smoke exposure (Willes *et al.*, 1991). Increased responsiveness is also not due to an allergic, IgE-mediated, response to smoke. Subjects were challenged and nasal lavage samples analysed for the presence of selected mediators. No increase in histamine occurred, indicating that the response did not involve mast cell activation (Bascom, 1991a).

Biological variability in the response to c-fibre stimulation in humans has been demonstrated. Intranasal capsaicin administered to ETS-sensitive subjects was associated with increased symptoms of rhinorrhea compared to symptoms reported by ETS-nonsensitive subjects (Bascom *et al.*, 1991b), and other investigators have quantitated increased nasal discharge following capsaicin administration in subjects with a history of watery vasomotor rhinitis (Geppetti *et al.*, 1988; Stjarne, 1989). Recent data indicate that the response to c-fibre stimulation is qualitatively different in rodents and in humans, however. An increase in plasma proteins cannot be measured in nasal lavage fluid following tobacco smoke exposure (Bascom, 1991a) or capsaicin challenge (Bascom, 1991b). The congestive response in humans appears to be predominantly vasodilation.

Is the biological variability observed in the acute response at all relevant to the risk of chronic effects of smoke exposure? For tobacco smoke, the answer to this question is unknown. However, epidemiologic data suggest that there is a significant relationship between an individual's acute airways response to an irritant and susceptibility of the airways to chronic exposure. In Vancouver grain workers (Canada), declines in lung function across a working week (an acute response) were highly correlated with declines in lung function over six years (a chronic effect: Tabona *et al.*, 1984). This effect was observed independent of smoking status and the longitudinal declines in lung function were not correlated with positive immediate skin tests to common allergens, the presence and absence of respiratory symptoms or initial lung function. Studies using inbred mice could assess the relationship between susceptibility to acute and chronic effects of tobacco smoke exposure.

Chronic tobacco smoke exposure causes effects on numerous target cells in the lung. Exposure to smoke for several weeks was associated with an influx of neutrophils and macrophages in C57B16 mice but not F-344 rats (Gairola, 1986). Epithelial changes in similarly exposed rats were associated primarily, but not exclusively, with the particulate phase of the smoke (Coggins *et al.*, 1980). Morphological changes which have been demonstrated in chronic bronchitis include hypertrophy and hyperplasia of submucosal glands, patchy squamous metaplasia, and a mild neutrophil and lymphocytic infiltration in the mucus membrane (Snider, 1988). In small airways (< 2 mm in diameter) mucus plugging, goblet cell metaplasia,

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inflammation, increased smooth muscle and distortion due to fibrosis can be seen. Emphysema is characterized by irregular airspace enlargement.

Biological variability has clearly been demonstrated in the response to chronic effects of tobacco smoke exposure. Only one-fourth of all regular smokers die of smoking related disease and 15–20% of smokers develop airflow obstruction. Fletcher sketched the biologic variability in the development of airflow obstruction in his classic paper (Fletcher *et al.*, 1976). The key point he made was that some cigarette smokers demonstrated declines in lung function which are similar to nonsmokers, while other people (termed 'susceptible') demonstrate accelerated declines in lung function. Data of Burrows *et al.* (1979) show an increased skewing of the population distribution of forced expiratory volume in one second (FEV_1) in association with an increased cumulative dose of tobacco smoke. A substantial proportion of individuals remain well within normal limits.

A contribution of heredity to chronic lung disease has been suspected at least since the early 1800's (Buist, 1988). However, at present, there is only one clearly established genetic determinant of tobacco smoke-related lung disease: the α_1Pi deficiency and this defect can account for less than 10% of chronic obstructive pulmonary disease however (Buist, 1988). There is a substantial literature which shows familial aggregation for chronic obstructive pulmonary disease unrelated to α_1Pi deficiency (Faling, 1983).

A commentary by Kazazian outlined a framework for considering lung disease from a genetic perspective (Kazazian, 1976). Familial aggregation of chronic obstructive lung disease cannot be explained by a single mutation. His expectation is that there are 'several or many rare single-gene causes of emphysema' but that in the aggregate, a small percentage of emphysema cases are due to single gene disorders. Most cases of emphysema, he speculates, are due to the effects of cigarette smoke on persons carrying mutant genes, each of which increases their risk of developing the disease. These genes are thought to be frequent ($>1\%$ of all genes at a locus) since mutations are needed simultaneously at two or more loci for disease to develop.

One approach is therefore to seek strain differences in specific components of the acute physiological and inflammatory response to tobacco smoke. Strains with divergent responses can be used to determine the locus and inheritance pattern of these responses. Strains which display divergent acute physiological or inflammatory responses could be compared in chronic tobacco smoke exposure studies to look for evidence of susceptibility to chronic diseases such as bronchitis and emphysema. Cited below are examples of sites at which allelic variations in critical proteins could cause increased susceptibility to airway disease from tobacco smoke. Inhalation toxicological studies using inbred strains of mice which characterize the genetics of specific responses to tobacco smoke will almost certainly provide fundamental advances in our understanding of the basis for biological variability in the response to tobacco smoke in humans. One of the practical reasons for this is that extensive work has already identified genomic homologies between mice and humans.

The first possibility is that susceptibility occurs because one or more critical cells of susceptible individuals receive an increased dose of a toxic component of tobacco smoke. The nasal and tracheobronchial mucosa demonstrate marked uptake of a variety of xenobiotics. This uptake presumably occurs through the action of cell-associated proteins which are subject to allelic variation.

A second possibility is that increased susceptibility occurs because of a genetically determined augmented inflammatory response. Chronic exposure to comparable doses of tobacco smoke induced an alveolar neutrophil influx in male C57BL mice but not F-344 rats (Gairola, 1986). Kleeberger *et al.* (1990) have shown that the magnitude of the alveolar neutrophil influx after ozone exposure in inbred mice can be localized to a single gene. Tobacco smoke is rich in oxidants and one may hypothesize that the neutrophil response to smoke is determined at the loci described by Kleeberger *et al.* (1990). It is also possible that differential recruitment of neutrophils to the alveoli is related to susceptibility to emphysema.

A third possibility is that susceptibility occurs because an element of host defense is impaired. Antiproteases are an important component of pulmonary host defence. α_1Pi is a 52 KDa serum protein with approximately 30 phenotypes which inhibits several types of proteolytic enzymes. Individuals with the homozygous Pizz phenotype have a level of α_1Pi that is 10–20% of that found in persons with the Pim phenotype. Smokers with the homozygous Pizz phenotype develop emphysema at an early age. While this is a vivid example of the ability of a single genetic defect to affect the host response to tobacco smoke, it only accounts for a small proportion of tobacco smoke-related chronic lung disease.

Antioxidants are also likely to be important in protecting the respiratory tract against tobacco smoke, since tobacco smoke itself is rich in oxidants (Nakayama *et al.*, 1984) and increase superoxide production by alveolar macrophages (Gairola, 1986). Recent studies in bacteria have demonstrated dra-

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matic differences in host sensitivity to oxidants (Storz *et al.*, 1990). The sensitivity has been traced to a locus of nine oxidant-inducible proteins. Whether a similar family of proteins exists in humans, and their clinical relevance, has not yet been determined.

The so-called 'Dutch hypothesis' states that non-specific bronchial hyper-responsiveness is a risk factor for chronic obstructive pulmonary disease (Buist, 1988). While airway hyper-responsiveness can be acquired (as in occupational asthma), there is also evidence that it is an inherited trait (Townley *et al.*, 1986), which may represent a risk factor for the development of disease. At present it is unknown which of these possibilities explains the cholinergic hyper-responsiveness observed in smokers. Studies by Levitt *et al.* have demonstrated that airway reactivity to acetylcholine and 5-hydroxytryptamine in inbred strains of mice are attributable to distinct autosomal recessive genes (Levitt & Mitzner, 1988, 1989; Levitt *et al.*, 1990). This model could be used to determine whether hyper-reactive strains demonstrate increased susceptibility to chronic exposure to tobacco smoke.

Other mutations hypothesized by Kazazian (1976) to alter susceptibility to emphysema included mutations in the structural gene for elastin or other proteins of the lung parenchyma, mutations leading to an increased protease activity in alveolar macrophages, mutations producing decreased antiproteases in bronchial secretions, mutations that increased leukocyte elastase concentration or activity and mutations that structurally alter the bronchial tree or chest wall. This range of possibilities serves to emphasize the need for animal models to seek for mechanisms relevant for susceptibility to a range of non-neoplastic effects of tobacco smoke.

Tobacco effects research in the last two decades has profiled physiological abnormalities, inflammatory mediators and cells which can be found in cigarette smokers or in individuals or animals exposed to environmental tobacco smoke, oxidants or aldehydes. Biological variability is well recognized. Well-characterized inbred strains of mice can now be used to study the genetics of these physiological and inflammatory responses to tobacco smoke. The results of these studies will provide new insights into critical differences between people who do, or do not react to low levels of irritants in their environment and differences between smokers who do and do not become diseased.

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